## REMARKS

Claims 22, 39, 46, 48, 62, 78, 87, and 92 have been amended. New claims 108-116 have been added. Support for a method of detecting a target cell whereby an second antibody directed to a cell surface antigen is mixed with a cell mixture and whereby a magnetic particle is coated with a first antibody directed against the second antibody are used can be found, for example on pages 4 and 6. No new matter has been added.

Indefiniteness Rejection

Claims 22-25, 28-29, 33-40, 43, 46-48, 51, 59-62, 64, 66-67, 69, 71, 72, 75, 78, 79, 87-89, 92, 93, 96, 101, and 105-107 were rejected under 35 USC 112, second paragraph as being indefinite. Applicants traverse the rejection to the extent it is maintained.

Applicants assert that the claims, as amended, are definite. In making the claim amendments, Applicants addressed the Examiner's comments regarding indefiniteness. Withdrawal of the rejection is respectfully requested.

Obviousness Rejections

Claims 22-25, 28-29, 33-40, 43, 46-48, 51, 59-62, 64, 66-67, 71-71, 75, 78-79, 87-89, 92-93, 96, 101, and 105-107 have been rejected under 35 USC 103(a) as allegedly being obvious over Jensen (US 5,374,531) taken altogether with Hermentin et al. (US 5,095,097) or Ullman et al. (Us 5,536,644).

Claims 22-25, 28-29, 33, 37-38, 48, 51, 59-62, 64, 69, 101, and 105 have been rejected under 35 USC 103(a) as allegedly being obvious over Widder et al. (EP 016,552) in view of Connelly et al. (US 5,422,277).

Claims 22, 46-48, 78-79, 106, and 107 have been rejected under 35 USC 103(a) as allegedly being obvious over Widder et al. and Connelly et al. in view of Forrest et al. (US 4,659,678).

Claims 22, 46-48, 78-79, 106, and 107 have been rejected under 35 USC 103(a) as allegedly being obvious over Widder et al. and Connelly et al. in view of Kemmer et al. (J. Immunol. Methods, 1992) and Holmes et al. (WO 91/09938).

Applicants traverse these rejections to the extent that they are maintained.

The presently claimed invention now provides a method for detecting target cells in a cell mixture where antibodies recognizing cell membrane structures are not directly bound to paramagnetic beads. Instead these targeting antibodies are added to the mixed cell suspensions and the cells are washed to remove free antibody. Paramagnetic beads coated with antibodies or fragments directed against the primary cell targeting antibodies are incubating with the washed cells and the target cells are isolated with the use of a magnet. The thus enriched cells are then visually examined with, for example, a microscope where the target cells with bound beads (rosettes) can be recognized as rosettes and counted.

Applicants assert that none of the cited references, alone or in combination, teach or suggest a method of detecting target cells according to the claimed invention. The detection of a specific target cell in a complex mixture is not taught or suggested by the combination of the cited references. The level of sensitivity required to detect a specific target cell in a complex mixture could not be achieved by the teachings of the cited references, either alone or in combination. Certainly, the level of sensitivity required by, for example, by claims 92, 110, 114, and 116 could not be achieved by the combination of the cited references. Withdrawal of the rejection is respectfully requested.

## CONCLUSION

In view of the remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Title: | METHOD FOR DETECTION OF SPECIFIC TARGET CELLS IN SPECIALIZED OR MIXED CELL POPULATION AND SOLUTIONS CONTAINING MIXED CELL POPULATIONS | | |

MARKED UP COPY OF CLAIMS SHOWING AMENDMENTS

22. (amended) A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a [single] cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

a. coating paramagnetic particles or beads with a first [monoclonal] antibody or antibody fragment directed against [a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture] a second antibody or antibody fragment;

b. incubating the second antibody or antibody fragment with the cell mixture to bind the antibody or antibody fragment with the target cell, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture ;

c. washing the cell mixture to remove unbound second antibody or antibody fragment;

[b.] d. mixing the coated paramagnetic particles or beads with the washed cell mixture [cell suspension containing the target-cells];

[c.] e. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and

[d.] f. [quantitating] visually detecting the target cell-bead rosettes after incubation.

39. (amended) The method of claim 22, wherein the monoclonal antibody or antibody fragment is directed against fibronectin receptor, β-integrin, vitronectin receptor, αγß3-integrin, P-seletin[,] including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, $Le^y$, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2[,] including HER2, transferin receptor, TNF-receptor, [high] molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope[,] including cluster 2 epithelial antigen, MUC-1 antigen[,] including DF3-epitope[,] and gp290kD, prostate [high] molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, $ß_2$-microglobulin, Apo-1 epitope, or pan-human cell antigen.

46. (amended) A kit for performing the method of claim 22, the kit comprising:

a. a specific monoclonal antibody or antibody fragment directed to an antigen on a target-cell, which monoclonal antibody or fragment is [effective for] capable of coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

c. a second specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;

wherein said second antibody or antibody fragment is conjugated to a detectable label.

48. (amended) A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a [single] cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

a. coating paramagnetic particles or beads with a first antibody directed against [an Fc-portion of] a second [monoclonal] antibody or antibody fragment;

b. incubating the second antibody or antibody fragment with the cell mixture to bind the antibody or antibody fragment with the target cell, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture;

c. washing the cell mixture to remove unbound second antibody or antibody fragment;

b. mixing the coated paramagnetic particles with the [second monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target cell and the cell suspension] washed cell mixture

c. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and

e. [quantitating] visually detecting the target cell-bead rosettes [after incubation].

78. (amended) A kit for performing the method of claim [48] 111, the kit comprising:

a. a first monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell;

b. a second antibody directed against an Fc-portion of the first monoclonal antibody or fragment thereof;

c. a paramagnetic particle or bead; and

d. a labeled third specific monoclonal antibody directed against an antigen or a receptor within or on the target cell.

62. (amended) The method of claim 48, wherein when the density of target-cells is low, or when the ratio of target cell/total cells in the cell mixture is [low (]≤ 1%[)], the method further comprises after incubating, applying a magnetic field to separate out the target cell-bead rosettes.

87. (amended) A method for detecting tumor cells in a cell suspension of mixed cell population or in a [single] cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, comprising:

a) coating paramagnetic particles with a first antibody or fragment directed against a second a tumor-specific monoclonal antibody or fragment;

b) incubating the second tumor specific antibody with the cell suspension to allow the second tumor specific antibody to bind the tumor cells;

c) washing the cell suspension to remove unbound second antibody or antibody fragment;

[a)] d) coating paramagnetic particles with a tumor-specific monoclonal antibody or fragment thereof;

[b)] e) mixing the coated paramagnetic particles with the cell suspension;

[c)] f) incubating the mixture at about 4°C under gentle rotation until tumor cell-bead rosettes are formed; and

[d)] g) [quantitating the number of] visually detecting the tumor cell-bead rosettes [which includes separating out the tumor cell-bead rosettes].

92. (amended) A method of detecting metastatic cancer cells in a suspension of a mixed cell population or in a single cell suspension from a solid tissue when the metastatic cancer cells are present at less than 1% of the cell suspension, the method comprising the steps of:

a) coating paramagnetic particles with a first antibody or fragment thereof directed against a second a cancer-specific monoclonal antibody or fragment;

b) incubating the second tumor specific antibody with the cell suspension to allow the second tumor specific antibody to bind the tumor cells;

c) washing the cell suspension to remove unbound second antibody or antibody fragment;

[b)] d) mixing the coated paramagnetic particles or beads with the cell suspension;

[c)] e) incubating the mixture under gentle rotation at about 4°C until tumor cell-bead rosettes are formed;

[d)] e) applying a magnetic field to separate out the tumor cell-bead rosettes; and

[e)] f) [quantitating] visually detecting the tumor cell-bead rosettes [after separation].

New claims 109-116 have also been added.